# SOME QUANTITATIVE PARAMETERS OF THE VASCULAR NETWORK OF EXPERIMENTAL TRANSPLANTABLE BRAIN TUMORS IN RATS

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Dependence of growth of a tumor on the degree of its blood supply is widely known and much attention has been devoted to the study of this problem in oncology. It has been shown that the fastest proliferating zones of a tumor usually coincide with areas of densest vascularization [1, 7, 14]. Spreading of tumor cells often takes place directly along the course of blood vessels [10, 12]. Metastasization of tumors also is connected with blood supply [5, 9]. Most attention has been paid in the literature to the study of mechanisms of new blood vessel formation: the angiogenic factor of tumor cells [8, 11], the topography of the vascular system of the tumor [1, 2]. Since tumor blood vessels constitute basically a network of the microcirculation, their essential cellular substrate consists of endothelial cells. The few investigations which have been devoted to the study of tumor blood vessels [10, 13, 15] have been concerned mainly with the rate of renewal of this population. Meanwhile much interest is attached to quantitative parameters of the vascular network such as, for example, the degree of vascularization, the area of cross section of vessels of the microcirculatory network, and the density of their endothelial lining, which together may perhaps determine the functional capacity of the newly formed tumor vessels.

The aim of the present investigation was to study certain quantitative parameters of the vascular network and the endothelial cell population in transplantable brain tumors varying in their degree of malignancy.

## EXPERIMENTAL METHOD

Minced fragments of tissue of malignant gliomas of strains 101-8 and 11-9-2, and also a neurinoma of strain 46-1 (approximately 5-8 million cells), obtained at the Research Institute of Human Morphology, Academy of Medical Sciences of the USSR [6], were implanted intracerebrally into Wistar rats. The degree of malignancy of the tumor was judged by the average survival time of the inoculated animals. It averaged 14 days for strain 101-8, 28.5 ± 1.4 days for strain 11-9-2, and 34.5 ± 2.3 days for neurinoma 46-1. At different times growth of the tumors the degree of development, topography, and characteristic features of their vascular system were studied. For this purpose angiography was carried out by intracardiac injection of 8-10 ml of ink with 4% gelatin under pentobarbital anesthesia (60 mg/kg). The brain was removed and unstained celloidin frontal sections were cut to a thickness of 15 $\mu$. The next stage of the investigation consisted of computer analysis of brain sections scanned on the PAK apparatus [3]. The total number of fields of vision occupied by the tumor was counted along a section with the greatest diameter of the tumor node, and the mean area of cross section of all vessels in one field of vision in a given section, the number of vessels, the percentage of fields of vision with hemorrhages relative to the total number of fields occupied by the tumor, and also the percentage of fields of vision occupied by necrotic areas of tumor in a given section, were calculated. It must be pointed out that with the methods used (computer analysis, luminescence-histochemistry) it was impossible to distinguish between capillaries, arterioles, and venules; indeed, it is difficult to do this in relation to tumor vessels. The term vessels is therefore taken to mean all three elements of the microcirculatory bed mentioned above. Meanwhile it was shown in [16] that

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the overwhelming majority of small blood vessels in the rat brain from 2 to 32 $\mu$A in diameter consists of capillaries 4-8 $\mu$A in diameter. The term "mean size of vessels" later in this paper will be taken to mean not the diameter of a vessel but the total area of cross section of individual segments in a scanned frontal brain section. The population of endothelial cells was studied by a method based on their ability to accumulate biogenic amines, which can be detected fluorescence-histochemically [4]. In order to accumulate dopamine, in particular, before decapitation the animals were given an injection of its precursor L-dopa (100 mg/kg) and the monoamine-oxidase inhibitor iproniazid (100 mg/kg). The brain was removed and quickly frozen in liquid nitrogen, freeze-dried, and treated in formaldehyde vapor for 1 h at +86°C. The brain was embedded in paraffin wax in vacuo and unstained paraffin sections 15 $\mu$ thick were prepared for luminescence microscopy. The number of endothelial cells per length of longitudinal section of the vessel was counted on a "Fluoval-2" microscope. Since dopamine is located in endothelial cells in the form of a compact fluorescent spot, the distance between neighboring spots along a segment of the vessels was counted by means of an ocular micrometer, and the density of the endothelial cells could thus be determined.

## EXPERIMENTAL RESULTS

The morphological picture of the tumor nodes of both gliomas (strains 101-8 and 11-9-2) was almost identical, but was very nonhomogeneous, resembling "marble" in character. Fields of continuous accumulation of weakly differentiated tumor cells alternated with areas of tissue destruction and lacunar hemorrhages. The tumor cells spread by infiltration mainly diffusely, but sometimes along blood vessels in the form of sleeves.

In preparations of the neurinoma (strain 46-1) a more homogeneous picture was observed. There were very few areas of destruction. Hemorrhages were present, but less frequently than in gliomas. The tumor cells spread expansively more often than in gliomas, and formed perivascular sleeves. Within the tumor node, concentrations of tumor cells were not as chaotic as in the gliomas, but were in some sort of order, forming structures of bands and loops.

Conforming to the rate of growth of the test tumor strains, observations on development of the vascular network; were made on the 4th, 7th, and 10th days for glioma 101-8, on the 5th, 10th, 16th, and 20th days for glioma 11-9-2, and on the 10th and 20th days for neurinoma 46-1. During this time the tumor cells of the first two strains increased in size on average by about 3 and 2.5 times, whereas the neurinoma virtually did not grow in size.

The degree of malignancy of the tumors leading to death of the animals was studied with the aid of the development of hemorrhages as the criterion. In strain 101-8, lesions of this type reached a maximum on the 7th day, whereas in the more slowly growing strain 11-9-2, the maximum was reached on the 20th day. The neurinoma had the lowest percentage of this type of pathology at the end of the period of observation. It will be recalled that the hemorrhages observed in the sections could have arisen not only spontaneously, but also under the influence of a sharp rise of intravascular pressure due to injection of a considerable volume of ink solution into the blood stream.

The pattern of arrangement and external appearance of the vessels was roughly the same in both strains of gliomas. The vessels were distributed unevenly. In some places single vessels were seen in a large area of tumor tissue, but in others concentration of the vessels was observed in the form of dense bundles. These bundles often, but not always, were located in the peripheral zone of the tumor. Most vessels of all tumors were modified in shape: convoluted, with uneven and, as it were, eroded contours of their lumen. This phenomenon was most marked in neurinoma 46-1, but vascular bundles were much less commonly seen, on the other hand.

It will be clear from Table 1 that the number of vessels per field of vision in all the tumors was less than in the control, namely the number of vessels in an area of the healthy hemisphere corresponding to the tumor. When this control was compared with the intact control, the number of vessels on the healthy side of the tumor-affected brain as a rule exceeded the normal value in the case of both gliomas, and was close to it in the case of the neurinoma. It is difficult to assume the secretion of different amounts of angiogenic factor, for the degree of vascularization of the neurinoma and gliomas was about equal in the case of strain 11-9-2, and actually less in the case of 101-8. In glial tumors, with a tendency toward destruction and hemorrhage, an inflammatory reaction evidently took place on the healthy side. The possibility of a purely mechanical increase in density of the vessels on the healthy side due to compression by the tumor growing on the other side, likewise cannot be ruled out. No special difference could be observed in the dynamics of the density of vascularization of all the strains studied during our period of observation, for no evidence was found that the density of the vascular network depends on rates of growth or degree of malignancy of the tumor. It can therefore be tentatively suggested that development of the neoplasm itself requires a certain level of blood supply, which is achieved quite early during development of the tumor. A study of transplantable sarcoma S-45 ([8a], Chap. III) showed that in the earliest stages of tumor development, before the 3rd day after inoculation, the number of

TABLE 1. Characteristics of Vascular Network of Transplantable Tumors at Different Stages of Development, as Shown by Computer Analysis

| Strain of tumor | Time after inoculation, days | Size of tumor (number of fields of vision) | Per cent of fields of vision of tumor with hemorrhages | Number of vessels per field of vision | | Per cent of control | Mean size of vessel | | Per cent of control | Number of animals |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | in tumor | control (healthy side) | | in tumor | control (healthy side) | | |
| Glioma | 4 | 33,0±11 | 5,4±2,2 | 34,2±4,2 | 67,0±7,2 | 51 | 11,9±1,6 | 11,9±2,0 | 98 | 4 |
| | 7 | 41,7±7 | 21,5±7,7 | 26,5±3,0 | 66,1±6,4 | 40 | 16,0±1,5 | 12,0±1,0 | 135 | 6 |
| 101-8 | 10 | 76,0±18 | 21,1±5,3 | 24,5±6,0 | 64,1±10,6 | 38 | 17,1±2,0 | 9,4±2,2 | 180 | 6 |
| Glioma | 10 | 30,0±7 | 9,1±3,0 | 40,0±5,7 | 68,5±5,2 | 59 | 15,0±2,3 | 10,8±0,7 | 139 | 8 |
| 11-92 | 16 | 51,0±11 | 16,8±3,7 | 36,7±6,3 | 51,7±2,0 | 72 | 14,9±1,1 | 10,0±1,0 | 149 | 8 |
| | 20 | 63,0±11 | 40,2±8,2 | 36,7±6,4 | 64,3±3,2 | 58 | 14,2±3,1 | 10,4±2,3 | 136 | 8 |
| Neurinoma | 10 | 49,0±4 | 8,1±2,5 | 39,4±4,1 | 48,7±1,8 | 80 | 11,3±1,0 | 9,6±1,0 | 118 | 6 |
| 46-1 | 20 | 33,0±9 | 12,6±9,2 | 29,4±3,4 | 59,5±4,7 | 50 | 14,7±2,4 | 12,0±1,0 | 123 | 5 |
| Intact control | | | | | 54,0±2,8 | | | 19,0±1,3 | | |

TABLE 2. Linear Density of Endothelial Cells in Vessels of Different Tumors and in Control as Shown or Histochemical Analysis

| Object | Time of observation, days | Number of animals | Linear density of endothelial cells | | |
|---|---|---|---|---|---|
| | | | pathological tissue | peripheral zone | healthy tissue |
| Intact control | | 14 | | | 31,7±0,7 |
| Control with trephining of the skull* | 5—7 | 11 | 33,3±1,6 | 33,6±1,2 | 34,2±1,4 |
| Neurinoma 46-1 | 15—20 | 13 | 27,2±2,0 | 31,0±1,3 | 33,6±1,2 |
| Glioma 11-92 | 14 | 15 | 25,5±1,8 | 30,6±1,4 | 34,0±0,7 |
| Glioma 101-8 | 7 | 17 | 25,4±0,7 | 32,2±0,8 | 33,0±0,6 |

*Region of reactive inflammation around site of trephining and puncture of brain tissue without implantation of tumor (mock inoculation)

vessels in the tumor exceeds normal, but later it decreases and falls below the normal level. It was shown in [15] that the mitotic index and labeling index of tumor cells decrease with increasing distance from the vessels. This is evidence that the role of the vessels in tumor cell proliferation is the same for different types of tumors.

The mean area of section of vessels in the tumor was a little greater than on the healthy side of the brain. Meanwhile both these dimensions are significantly less than the normal value, possibly on account of the compression factor or reflex spasm.

If the appearance of hemorrhages is taken as the criterion, it can be seen that the two gliomas, while differing with respect to the survival time of the animals, nevertheless have about equal density of their blood vessels, whereas in vessels of the neurinoma at the same stage of development of the tumor, the tendency toward fragmentation was rather less marked (although the scatter of the data relating to this parameter is wide). This suggested a possible qualitative difference in the structure of blood vessels in different tumors. To shed light on this problem the linear density of the endothelial cells (the number of cells per unit length of thee vessel) was studied.

It will be clear from Table 2 that endothelial cells of vessels newly formed in the substance of the tumor are less densely arranged than in normal vessels, although no difference was observed in the linear density in different strains. The less dense distribution of endothelial cells along the length of the tumor vessels, in our view, may be due both to partial injury to the cells by toxins arising iron disintegrating areas of the tumor, and to an unbalanced, accelerated growth of the vessels in the tumor tissue under the influence of angiogenic factors. We did not make a special study of the degree of erosion of the endothelial cells in the tumor vessels, bud the increased permeability of these vessels, which is well known, does not allow the presence of defects in the endothelial layer to be ruled out. The presence of such defects in vessels of a hepatoma have been reported in [14]. The about equal linear density of the endothelial cells in all strains, accompanied by a greater tendency for vessels in gliomas to rupture, especially in strain 101-8, may perhaps be explained by the choice of vessels for counting the cells, because the most injured vessels, along the linear regions of which it was difficult to find more than one fluorescent spot, were not subjected to examination. In the peripheral zone of the tumor, from which invasion of the vessels takes place, the linear density was about within normal limits. Vessels in the zone of the post-traumatic inflammatory reaction, however, had a rather higher linear cell density than normally. The same is true also of vessels of the healthy cerebral hemisphere in animals with tumors. On the one

hand, this is further confirmation of the presence of an inflammatory reaction in vessels of the healthy part of the brain, affected by the tumor, and on the other hand, it is evidence that the inflammatory vascular reaction is accompanied not only by an increase in the number of vessels, but also by an increase in the linear density of endothelial cells in the vessels. No clear relationship could be observed under our experimental conditions between the varied tendency of the strains of the test tumors to develop hemorrhages and the linear density of the endothelium in the vessels of these strains. On the whole, however, the integrity of the vessels, relative to the criterion of rupture, correlates with a lower linear density of the endothelial cells in tumor vessels than in the vessels of normal tissues.

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